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Review

Cartilage: A new parameter for the determination of the postmortem interval?

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ABSTRACT

The determination of the time of death or the postmortem interval (PMI) is one of the most important and frequently asked questions in forensic medicine. The methods used for PMI determination are based largely on early and late postmortem changes. The determination of the PMI during the late postmortem changes is based primarily on a subjective assessment and is less precise due to the lack of objective methods. Different studies have presented a gradual decrease in chondrocytes' viability but these researches did not answer the question whether we can use the decrease of chondrocytes' viability for an objective PMI determination. The structure and anatomical location of the cartilage together with its mechanical, physical and chemical properties enable chondrocytes to survive for several weeks after the individual's death, and give cartilage the attributes of a compartment. Therefore, cartilage could be a new parameter for PMI determination. This idea had been partially confirmed by a few *in vitro* studies. The next step in testing this idea should be an extensive *in corpore* study.

1. Introduction

The determination of the time of death or postmortem interval (PMI) is one of the most important and most frequently asked questions in forensic practice. Despite the fact that the determination of PMI is among the most researched areas of forensic medicine, the results of these studies are relatively modest.

Eighteen years ago a group of leading experts in the field of forensic medicine collected the results of these studies and published the first book on the topic of PMI determining,[1] especially during the development of early postmortem changes, such as body cooling, *rigor mortis*, and *livores mortis*, whose dynamics help to determine the PMI, but only during the first 24–36 h after death or until the postmortem changes due to putrefaction. In 2002, the same group of experts prepared the second edition,[2] which was a reprint of the first edition with an added chapter on determining the PMI based on the stage of digestion after a meal and gastric emptying. This was particularly emphasized by other experts in the field of forensic medicine,[3,4] but in the foreword to the second edition Knight explained that in seven years since the first edition relatively few original researches of PMI determination had been published. Even in the last decade important progress on this issue has not been reported.[5–8]

Among the other methods which could be used for PMI determination in the first hours and days after death are the assessment of the supravital reactivity of the skeletal muscles after mechanical or electrical stimulation, and the chemical or histological changes in the compartments (parts of the body that are anatomically separate from their surroundings and thus less affected by putrefaction) mainly changing the concentration of potassium in the vitreous humor.[9–12] According to the available databases, the last survey of changes in the compartments for PMI determination considered the postmortal decline in the number of odontoblasts in the dental pulp.[13] Histological and biochemical changes in other organs, such as in the heart or liver, were also studied for PMI determination but their practical use is questionable.[14,15]

However, Henssge and Madea pointed out that every forthcoming parameter for the practical relevance in evaluating the PMI should fulfill the following criteria: the quantitative measurement, mathematical description, taking into account the influencing factors quantitatively, a declaration of the precision and the proof of precision on independent material.[16]

The intention of this paper was to answer whether cartilage could be determined as a compartment, and if cartilage could be used for the determination of PMI. Therefore, in the paper there were described the particular attributes of the cartilage and the facts that supported this idea.

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2. Could cartilage be determined as a compartment?

Cartilage is a highly specialized connective tissue that acts as a support tissue which consists entirely of cartilage cells (chondrocytes), and a large amount of intercellular mass called the extracellular matrix (ECM). There are three types of cartilage:[17–21]

- hyaline cartilage (the surface of articular ends, parts of the nose and respiratory branches, and the ends of the ribs)
- elastic cartilage (the outer ear, Eustachian tube, and epiglottis)
- fibrocartilage (the mandibular and sternoclavicular joint, intervertebral discs, symphysis pubis, menisci, and tendon insertions)

Hyaline cartilage in the synovial joints is a highly specialized tissue that is adapted to operate in a highly stressed environment, and usually without malfunctioning during an individual's entire life. The main tasks of the joint cartilage are the distribution of loads over a wider surface, and minimizing articular friction and damage during articular movements.[22–25] Articular cartilage is an isolated tissue without nerves or blood and lymphatic vessels. Cartilage such as in the ribs is exceptionally minimally vascularized. The chondrocytes in the articular cartilage are supplied only by diffusion through the ECM. These elements limit the final thickness of the cartilage, which is typically 1–6 mm.[19,25]

2.1. Cartilage cells (chondrocytes)

The cartilage solely contains a homogeneous cell population of chondrocytes. The density of the cells in the cartilage tissue is lower than in any other tissue and occupies up to 10% of the volume.[26]

The density and distribution of chondrocytes in the uncalcified part of the articular cartilage is different (Fig. 1). In the superficial tangential zone (layer) there is a high density of oval-shaped chondrocytes, whose longer axis is parallel to the surface of the cartilage. In the middle zone (intermediate layer) there are round chondrocytes, which are randomly distributed and diluted. The chondrocytes in the deep zone are rounded and arranged in the form of pillars, which are vertical to the boundary (tidemark) between the uncalcified and calcified part of the cartilage. Chondrocytes are supposed to act differently in the different layers.[25,27,28]

Chondrocytes, though small in number, produce, secrete, set up and maintain the organic parts of the ECM, whose ingredients are in a constant state of dynamic equilibrium, homeostasis (continuous “turn over”), which means that the state of the ECM depends entirely on the chondrocytes. In comparison to other cells, chondrocytes live in modest conditions: having a low supply of nutrients, reduced oxygen concentration and acidosis. Increased oxygen concentration even inhibits chondrocyte functioning, on the contrary hypoxia induces collagen crosslinking and enhances the mechanical properties of the articular cartilage.[17–19,25,28–33]

Medical conditions such as osteoarthritis increase the permeability of the cartilage which lowers its resistance to pressure. The changed load carrying capacity has an influence on the chondrocytes and forms an imbalance between the chondrocytes' anabolic and catabolic activities, which leads to a vicious circle of progressive cartilage degeneration.[25,34,35]

2.2. Extracellular matrix (ECM)

The ECM of the articular cartilage forms a dense network of collagen fibers, especially type II with small amounts of collagen types V, VI, IX, X and XI, which are encased in a ground substance, a concentrated solution of proteoglycan (PG).[30,32,36–38]

Parts of the articular cartilage are usually: collagen (15–22%), PG (4 and 7%), and the rest is water with inorganic salts and a small amount of matrix proteins, glycoproteins and lipids. Collagen fibers and the PGs are able to create a highly robust network structure by themselves but the real biomechanical properties of these structures are only shown with water.[24,28,39–46]

2.2.1. Collagen

Collagen fibers in hyaline cartilage are usually arranged in a three-dimensional structure that resembles felt. Collagen is secreted in the ECM as tropocollagen, which is the basic biological unit made of three procollagen polypeptide chains, alpha helices, twisted to the left, which are further wrapped one around the other to the right triple helix. Tropocollagen rod-shaped molecules polymerize into large collagen fibrils.[17,19,36,37]

The most important mechanical feature of the collagen fibers is their resistance to elongation, which is enabled by an arrangement of tropocollagen molecules, wherein each tropocollagen molecule overlaps the other molecules by one quarter of its length. This provides a striation to the collagen fibers. Covalent cross-links between the tropocollagen molecules further contribute to the stretching resistance of the collagen fibers.[25,37]

The collagen fibers of the articular cartilage are arranged in a non-homogenous way and give it the appearance of tissue with three layers, zones (Fig. 1). The superficial, tangential zone is 10–20% of the total thickness of the articular cartilage. It contains densely packed fibers, which run parallel to the smooth surface of the articular cartilage, and is called *lamina splendens*. In the middle zone, which is 40–60% of the total articular cartilage thickness, the collagen fibers are orientated in all directions, homogeneously dispersed, and less densely packed with a greater spacing between

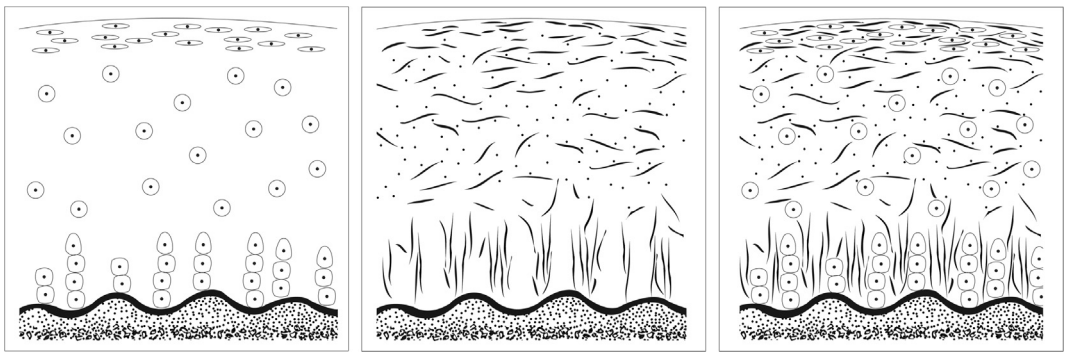

Fig. 1. Histological structure of articular cartilage. *Left*: position and shape of chondrocytes in layers (zones). *Middle*: distribution of fibers in the extracellular matrix and zones. *Right*: chondrocytes and the extracellular matrix together.

the fibers. Therefore, this layer is able to retain large amounts of highly concentrated PGs and water. In the deep zone, which is approximately 30% of the total thickness of the articular cartilage, collagen fibers form bundles crossing the border between the cartilage and the calcified parts below, so that they attach the cartilage to the underlying bone. The previously described arrangement of chondrocytes corresponds to this organization in the zones. Such a structure provides a more equal loading of the cartilage in the joint[25,28,46–52]

2.2.2. Ground substance

The ground substance is a semi-liquid gel, in which the dry fraction (Fig. 2) is composed of glycosaminoglycans (GAGs) in the form of hyaluronic acid (HA) and PGs.[19]

2.2.2.1. Proteoglycan (PG). Proteoglycan (PG) is the major protein-polysaccharide molecule, which consists of a protein core to which is attached one or more types of GAG (Fig. 2). GAGs are long, unbranched polysaccharide chains composed of repeating disaccharide units in which are usually uronic acid and amino sugar (N-acetyl glucosamine or N-acetyl galactosamine). A GAG molecule is acidic and negatively charged due to the presence of hydroxyl, carboxyl and sulfate groups in the disaccharide units.[19,30,31,53]

PG molecules are also called aggrecans because of their ability to associate (aggregate) with giant molecules. Aggrecans form two kinds of GAGs: chondroitin sulfate (CS), and keratan sulfate (KS). Each CS chain contains 25 to 30 disaccharide units and the shorter KS contains 13 disaccharide units. The central part of the aggrecan is an about 200 nm long protein to which are bound approximately 150 GAG chains and oligosaccharides, which are covalently attached. The core protein contains three globular structures with specific functions, including binding to the hyaluronic acid (HA), which is the "backbone" of the molecule compound that resembles a bottle brush (Fig. 2). HA may be an extremely long molecule, up to 4 μm. HA is a chain of disaccharides without sulfate residues. Although the cartilage contains a small quantity of HAs it has an extremely important role in the structure of the ground substance. The PG aggregates stabilized matrix determines its capacity, and allow its compression properties. The chemical links between the PG, HA and collagen fibers enable the transmission of high pressures, the link between the GAGs and the collagen fibers provide the strength of the cartilage.[18,30,31,54–57]

There are two basic populations of aggrecans. The first population is present throughout life and is rich in CS. Another PG is rich in KS and is present only in adult cartilage. The amount of water and CS in the cartilage progressively reduces with aging. On the contrary, KS, which is present only in small quantities at birth, increases with aging. The ratio CS/KS is 10:1 at birth, in adults it is 2:1. Also, in some other studies, changes to the articular cartilage are associated with aging, particularly as a result of the increasing load on the cartilage due to the increasing body weight and the different speed of collagen turnover in the cartilage remodeling as a function of the distance from the articular surface or bone (zonal variations) in the different ages.[28,31,40,58–69]

PGs are unevenly distributed in the matrix. As was previously mentioned, the highest PG concentration is usually in the middle zone, and the lower concentration is in the superficial and deep layers. The biochemical consequences of this uneven distribution are seen in the different levels of fluid retention and electrolyte distribution in the cartilage zones and pericellular, territorial or interterritorial ECM.[28,35,40,60,70–74]

2.2.2.2. Water as part of the ECM. GAG molecules are not flexible enough to establish globular aggregates but remain apart and form a large volume with a relatively low mass. Additionally, because of the electric charge in a number of molecular groups, GAG is highly hydrophilic so attracts a huge quantity of water and positive ions, particularly sodium. Therefore, in this fluid there are numerous free cations (e.g. Na^+, K^+ and Ca^{2+}), which strongly influence the mechanical, chemical, and physical properties of the cartilage.[19,28,40,59,75–77]

PGs can absorb the volume of a solution which is 50 times greater than their dry weight. Water represents 60–78% of the net weight of the hyaline cartilage. Just below the surface of the articular cartilage is the highest percentage of water (~80%), which linearly decreases to approximately 65% in the deeper parts of the cartilage. A smaller percentage of the water in the cartilage is inside the cells. About 30% of the water is firmly attached to the collagen fibers. The largest amount of water is in the space between the fibers in the ECM, and moves freely because of the gradient during loading, increased pressure or the electrochemical forces in the tissue. The water in the articular cartilage is essential for the avascular tissue as it allows the movement of gases and nutrients between the cells, the excretion and removal of waste products, and secretions of the synovial fluid, which is rich in nutrients.[17,18,28,40,58,59,72,76,78–81]

It can be concluded that the ECM is not only something that fills in the space around the cells but also gives mechanical support to the cells, acts as a store of water and solutes, even molecules that regulate the relationship between themselves, between the ECM and the cells, and between the cells by providing cell polarity, by using the cell receptors from the family of integrins, and by the growth factors which control the degree of differentiation, proliferation and apoptosis of the cells.[32,35,82]

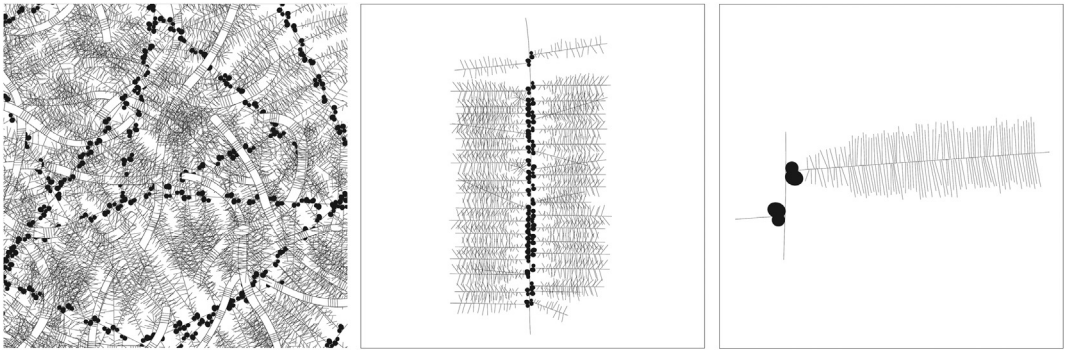

Fig. 2. The molecular structure of the extracellular matrix. *Left*: "felt-like" collagen fibers with glycosaminoglycans, forming a permeable matrix with a 6 nm average diameter of the pores. *Middle*: hyaluronic acid with proteoglycans resembling a bottle brush. *Right*: proteoglycan (aggrecan) with globular proteins on hyaluronic acid and disaccharide chains of chondroitin sulfate and keratan sulfate.

3. Cartilage as a compartment

Macromolecules of the ECM form a porous, permeable matrix which is reinforced with fibers, and has the mechanical properties of a solid structure, despite being filled with water. Such a structure enables the resistance of the cartilage, particularly articular cartilage, to pressure and stretching. At the same time the pores, which have an average diameter of 6 nm in a normal cartilage,[24,40,83–86] are the major barrier to microorganisms, even saprophytics, which do not need a living host for their existence. The smallest diameter of these bacteria, which are smaller than the other saprophytic microorganisms, is usually between 0.3 and 0.5 μm, and the smallest dimension of some elongated or spiral bacteria could be 0.1 μm.[87,88] Even the smallest dimension of nanobacteria, which is between 0.05 and 0.2 μm, is much bigger than the pores in a normal cartilage.[89,90]

The properties of the articular cartilage are also provided by the chemical structure and physical interaction between the PG aggregates in the ECM. The closely positioned sulfate and carboxyl groups in the CS and KS chains dissociate in a solution of physiological pH. The result is a high concentration of negative charges, which induces strong repulsive molecular and intermolecular electrical forces. When the tissue is immersed in a saline solution the sum of these forces is the same as the Donnan osmotic pressure.[53,70,75–77,91,92]

Additionally, it is necessary to take in consideration that the torso is a source of saprophytic microorganisms, so the anatomical position of some joints could have an advantage in the use of their cartilage compared to the other sources of cartilage.

Therefore, the anatomical, morphological, mechanical, physical and chemical properties of cartilage, in particular of the articular cartilage, are those with which the cartilage could be determined as a compartment. The properties of articular cartilage which define it as a compartment are summarized in Table 1.

4. Could cartilage be used for the determination of PMI?

The modest requirements for nutrients and oxygen of a small number of chondrocytes in an abundant ECM with different solutes, which diffuse to the chondrocytes after clinical death, allow the survival of the chondrocytes several days after the individual's death. In addition to the modest requirements, and the resilience of the chondrocytes to the oxygen deficiency and acidosis, dense fibers of the ECM provide an extra protection to the chondrocytes against microorganisms, mainly saprophytic bacteria that spread postmortem.[25,29,32,87,88,93–96]

Table 1
Attributes of articular cartilage defining it as a compartment.

Substrate	Attributes
Articular cartilage	Distance from the torso – the source of the saprophytic microorganisms tissue without vessels and nerves
Ground substance	Semi-liquid gel
- Extracellular matrix	Tenacious and dense but porous and permeable matrix pores with 6 nm average diameter obstruct the spread of microorganisms highly hydrophilic
- Water	60 to 78% of the net weight of the hyaline cartilage storage for gases, nutrients, proteins, lipids, electrolytes diffusion of solutes (gases, nutrients, metabolic waste, …)
Chondrocytes	Homogeneous cell population A small number of cells in the tissue (10% of the tissue volume) Modest requirements for oxygen and nutrients Low cellular activity, proliferation exceptionally Functioning in an environment with a lower pH

Researches on the chondrocytes long-term survival were made mainly because of the chondrocytes' cultivation or their conservation in osteochondral samples for clinical use, especially for articular cartilage transplantation. In these studies, the difference in the proportion of chondrocytes that survived during the time and at different temperatures was observed. Studies on human allografts showed that approximately 70% of the chondrocytes survived for one month, and that approximately 35% of the chondrocytes survived for two months if the samples were kept in the tissue banks at 4 °C, and under optimal conditions.[94–97]

According to the available databases only one study has analyzed the viability of chondrocytes in corpses, actually in the articular cartilage of the femoral part of the knee. It was found that almost 60% of chondrocytes survived six days, and almost 10% of chondrocytes survived 45 days in the corpses which were most of the time in an environment with temperatures around 4 °C. In the corpses it was found that during the summertime 70% of chondrocytes survived for two days, and 8% of chondrocytes survived for two weeks.[93]

Therefore, the gradual decline in the proportion of chondrocytes that survive clearly demonstrated that chondrocytes viability was a function of time and temperature, which could be useful for the determination of PMI.

5. Discussion

The previously mentioned facts supported the idea that cartilage could be determined as a compartment and could be used for the determination of the PMI. The regularity in the gradual decrease in the chondrocytes' viability as a function of time and temperature could help in the objective determination of the time of an individual's death several weeks after the moment of dying.

However, a broader study of cartilage as a possible new parameter for PMI determination in forensic medicine has not been performed. Since the corpse is exposed to a wide variety of environmental conditions that cannot be controlled, it was necessary to perform a study in supervised *in vitro* conditions before an extensive *in corpore* study.

Nevertheless, even before the *in vitro* study it was necessary to standardize the procedures, and to optimize the conditions, so that they simulated the state in the dead body as close as was possible. This was finished with the few pilot studies. Two pilot studies revealed that the optimal place for sampling was the articular cartilage of the femoral part of the knee; the best method for sampling was harvesting by the mosaic-plasty coring instruments that enable osteochondral samples with the whole cartilage thickness, which was important as chondrocytes of different layers in the articular cartilage were differently active; and among the apparatuses used in the analysis the best were the confocal laser scanning microscope (CLSM) and the cell viability analyzer (CVA). The CLSM showed a slightly better reliability compared to the CVA, but due to the highly technical requirements and for price reasons the CLSM was confirmed as more suitable for basic research, and the CVA was confirmed as more suitable for routine cartilage analysis.[98,99] These pilot studies also revealed the necessity for designing an appropriate storage method and the media for *in vitro* storing of the harvested samples during the study which was done by the additional pilot studies but were not published separately. However, all these studies confirmed that chondrocyte viability decreased gradually and steadily over time, but with different speeds at different temperatures, even in conditions that simulate the state in a dead body. Additionally, these studies have revealed that the method of cartilage sampling is simple and it could be done even at the crime scene. After an 8–10 mm medial or lateral parapatellar skin incision has been performed and following entry into

the knee joint, the osteochondral samples could be harvested in a few minutes using the surgical instruments for the mosaic-plasty or osteochondral autograft transfer system (OATS) from the medial or lateral femoral flare of the trochlea. After transporting the samples, the CVA analysis could be finished and the answer about the PMI interval could be produced in less than 24 h[99,100]

Besides the attributes which define cartilage as a compartment (anatomical, morphological, mechanical, physical and chemical properties) for the design of an *in vitro* study it was also necessary to take into account that the ECM composition varies with the individual's age, hormones, systemic or local diseases, cartilage damage, and other kinds of stress that affect the responsiveness of the chondrocytes and the ECM.[29,32,82] These facts suggested that it was necessary to design an *in vitro* study with samples from uniform donors (same gender, age, and without disturbing elements such as diseases). The *in vitro* study, which considered all the previously mentioned information with the conditions that simulate the state in a dead body, additionally confirmed that over time the chondrocytes' viability decreased gradually and steadily, and with different speeds at different temperatures. Even more, there was a possibility for a retrograde although indirect estimation of the PMI with a 95% probability.[101] However, all the above-mentioned have provided the reference data for future studies of cartilage as a possible new parameter for the determination of the PMI.

The idea that cartilage could be a new parameter for the determination of the PMI should be tested through an extensive *in corpore* study. The most important antemortem variable which should be taken into consideration through an *in corpore* study is the degenerative changing of the cartilage as a result of diseases and aging. Despite the degenerative disorders of cartilage being quite common, it should not have a crucial impact on the idea of using cartilage for the determination of the PMI because this idea should be applied to the general population where degenerative changes of the knee cartilage are presented in a small part of the general population, and the anatomical places for harvesting cartilage samples are usually not affected (medial or lateral femoral flare of the trochlea). The most important diseases that cause articular degenerative changes are osteochondritis dissecans (OCD) and osteoarthritis (OA). The incidence of OCD is estimated at 0.015–0.030% in the general population and it is most frequently in the knee where the medial femoral condyle is involved in 80% of cases. The incidence of OA increases with aging. In the United States, it is estimated that 85% of people 75 years' old and older are afflicted. A European study estimated that the prevalence of OA is around 40% for persons 80 years' old and older. OA is thought to be more prevalent in the developed regions of the world. An interpolation of these data for the general population shows that a very small part of the general population has degenerative cartilage changes. Namely, the current demographic statistics of the general population in the more developed countries show that 8.1% of the population is 75 years' old and older, but in the less developed countries only 2.3% of the population are of the same age. Part of the older population is quickly reduced with aging in both areas: those who are 80 and more years' old in the more developed countries account for 4.7% and in the less developed countries this represents only 1.1% of the general population.[102–104]

However, the suggested method for the late PMI determination is based on the chondrocytes' live-dead ratio in the analyzed samples. Therefore, mathematically it is not important if the total number of chondrocytes has already been decreased in the moment of the individual's death. Anyway, in the accessible data there was no information on the chondrocytes live-dead ratio changing in the degenerated cartilage of the corpses during the several weeks after the individual's death, so we could not affirm this consideration without a research study. After an extensive *in corpore* study, which should also include the corpses with degenerative cartilage changes, it could be found whether cartilage degeneration affected the chondrocytes live-dead ratio changing or not. In the case of the regular relation between healthy and affected cartilage there could be included a corrective factor for the different levels of cartilage degeneration which could be used for recalculating the obtained result of the chondrocytes live-dead ratio as in Henssge's temperature-relating PMI nomogram.

6. Conclusion

Cartilage, especially articular cartilage, could be determined as a compartment. A chondrocytes' viability decreases gradually and steadily as a function of time and temperature, which could be useful for an objective determination of the PMI in the late postmortem period for several weeks. The idea that cartilage could be a new parameter for the determination of the PMI should be tested through an extensive *in corpore* study.

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Conflict of interest
There is no conflict of interest.

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